

Formation and Fate of Gaseous and Particulate Mutagens and Carcinogens in Real and Simulated Atmospheres

by James N. Pitts, Jr.*

The growing use of coal for heating and electric power generation and diesel engines in light duty motor vehicles will increase not only the existing atmospheric concentrations of criteria pollutants such as NO₂, SO₂, O₃ and fine particulates, but also the concentrations of a number of highly reactive gaseous copollutants such as HONO, HONO₂, PAN and the nitrate radical, NO₃. These gaseous noncriteria pollutants are of interest not only because of their roles in the chemistry of the "clean" and polluted troposphere, including "acid rain," but also because they may pose health risks disproportionate to their relatively low ambient concentrations, and through complex heterogeneous reactions, they may serve as precursors or catalysts in the formation of "nonclassical" particulate mutagens and carcinogens such as certain nitroarenes associated with combustion generated particulate polycyclic organic matter (POM). Results of research efforts to establish current ambient levels of these noncriteria pollutants and to develop an understanding of their sources, formation and sinks are reported here. First, long pathlength (≥ 1 km) infrared and UV-visible spectroscopic studies of ambient levels of gaseous HONO, NO₃, HONO₂, PAN, HCHO and HCOOH in southern California atmospheres are described, and data given on their ambient concentrations. Second, an integrated chemical/microbiological investigation is described. It is directed toward identifying the nature of direct-acting mutagens found in extracts of diesel and ambient POM, as well as those formed upon exposure of environmentally relevant PAH to simulated natural and polluted atmospheres. The identification of certain of these mutagens, including a newly identified class of mutagenic PAH-lactones is discussed, along with the mechanisms of their formation and fate in the natural and polluted troposphere.

Introduction

In the 1980s, an increased utilization of diesel engines in light duty motor vehicles (LDMV) (1,2) and of coal for power generation and heating (3) clearly will further tax the already heavy atmospheric burden of fine particulates and gaseous nitrogen and sulfur oxides (NO_x and SO_x) in major urban areas throughout the world.

Until recently, concern would have focused primarily on the health impacts associated with the additional emissions of the major criteria pollutants NO₂ and SO₂ and of certain polycyclic aromatic hydrocarbons (PAH) in the particulate polycyclic

organic matter (POM) believed responsible for its carcinogenicity in animals (4,5). Additionally, some attention would have been given to the fact that the activity [as well as the rates of transformation in cell cultures (6)] was significantly greater than could be accounted for by the amounts of well-known carcinogenic PAH, such as benzo(a)pyrene (BaP), determined to be in the POM extracts (2,5,7-15). This "excess carcinogenicity" implied that carcinogenic compounds, as yet uncharacterized, must also be present.

Today, yet another dimension has been added to the problem. Around 1975, with the development of the short-term Ames *Salmonella typhimurium* reversion assay for chemical mutagens, research on both ambient and primary POM accelerated rapidly, and it is now well known that extracts of ambient POM contain direct-acting (not requiring

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metabolic activation) frameshift mutagens (16-24). Furthermore, both these direct mutagens and the classical carcinogenic PAH such as BaP (which are promutagens and require activation by S9) are concentrated in respirable particulates $<1 \mu\text{m}$ in diameter (25-31). Similar considerations (i.e., direct mutagenicity in the small diameter range) apply to fine particles emitted by gasoline (32) and diesel engines (33), the combustion of coal for electric power generation (34) and the residential burning of wood (35). However (and this is the chemical crux of the present concern over POM from diesel exhaust), just as with the "excess carcinogens," with but a few exceptions (*vide infra*) the chemical structures of these direct-acting mutagens in combustion-generated POM are unknown, or at best only tentatively identified.

Therefore, while the present intense scrutiny being given combustion-generated particulates is both understandable and desirable, it is important to recognize that associated with increases of diesel POM and fly ash from coal combustion there will be a corresponding rise in ambient levels of noncriteria pollutants, both in the gaseous and liquid aerosol phases. Some of these, identified spectroscopically in ambient and simulated atmospheres, are shown in Tables 1 and 2.

Certain of these so-called "trace" species [e.g., gaseous nitrous and nitric acids (HONO and HONO_2)], while present at much lower ambient concentrations than the criteria pollutants [~ 0.01 -100 parts/ 10^9 (parts per billion, ppb) vs. ~ 0.05 -0.5 parts/ 10^6 (parts per million, ppm)], may pose significant ecological and/or health problems even today (see Table 1). If so, then future air quality impacts will

Table 1. Trace gaseous pollutants identified spectroscopically in ambient southern California air and approximate concentration ranges observed in light to severe episodes of photochemical air pollution.

Compound	Range of peak concentrations	Time of peak concentrations
Nitric Acid (HONO_2)	1-50 ppb	Midafternoon
Peroxyacetylnitrate (PAN)	1-40 ppb	Midafternoon
Nitrous acid (HONO)	1-8 ppb	Early morning (before sunrise)
Nitrate radical (NO_3)	5-350 ppt	Night
Formaldehyde (HCHO)	1-80 ppb	Midafternoon
Formic acid (HCOOH)	1-20 ppb	Midafternoon
Nitrogen dioxide (NO_2)	0.1-0.8 ppm	Morning/afternoon

Table 2. Gaseous nitrogenous species identified spectroscopically in simulated atmospheres, and approximate concentration ranges predicted for ambient urban photochemical air pollution episodes.

Compound	Range of peak concentrations	Time of peak concentrations
Peroxynitric acid (HO_2NO_2)	50-200 ppt	Midafternoon
Peroxyalkyl nitrates (RO_2NO_2)	10-50 ppt	Midafternoon
Dinitrogen pentoxide (N_2O_5)	100-1000 ppt	Late afternoon and early evening

become increasingly serious if their atmospheric roles and health effects are not understood and their ambient levels controlled.

It has been the long-time belief of this author that, either directly or through complex chemical and biological interactions, certain of these gaseous noncriteria pollutants may contribute to both the nononcogenic and oncogenic health impacts of primary exhaust emissions and of ambient smog (36-40). Therefore, they should not be overlooked in the current pressing search for new atmospheric mutagens and carcinogens in combustion-related POM. Indeed, through heterogeneous interactions with PAH, trace nitrogenous pollutants such as HONO , HONO_2 , NO_3 and peroxyacetyl nitrate (PAN) may well be responsible for the formation of certain mutagens and excess carcinogens.

Underscoring the present need to identify and quantify the chemical and biological roles of certain noncriteria gaseous pollutants concurrently with the spectrum of PAH derivatives (and other organics) deposited on the surface of POM are recent results reported by Pepelko at the October 1981 EPA Symposium in North Carolina (41). He summarized extensive EPA studies of the effects of whole diesel exhaust (gaseous and particulate emissions) on animals in the following terms:

"There was little evidence that inhalation of diesel exhaust (DE) resulted in the induction of tumors.—By contrast a wide variety of non-oncological effects were found."

These included: a decreased level of both voluntary activity and exercise tolerance, a detrimental effect on learning in adult rats exposed early in life to DE and evidence for delayed neural maturation. Additionally, he pointed out that:

"A variety of changes were detected in lungs" and "Resistance to infection decreased markedly after DE inhalation and clearance mechanisms were overwhelmed."

In conclusion Pepelko noted:

"The marked effects found in the non-oncogenic studies are likely due to the presence of vapor phase components

such as nitrogen oxides, aliphatic aldehydes, etc., which would be expected to show a much greater degree of bioavailability than potential carcinogens." He concluded that "further studies are needed to delineate threshold limit values and to isolate and identify components of exhaust responsible for producing these changes."

These latest findings are both fascinating and perplexing. Thus at the same conference, it was reported by Nesnow that, on the basis of their recent skin carcinogenesis studies, the potencies of extracts of diesel POM collected from several engines range from "equivalent to roofing tar to marginal," depending on the particular engine (42). Furthermore, in studies from several laboratories, it has now been well established that several nitroarenes isolated from diesel POM are powerful bacterial mutagens, and certain related nitro-PAH are believed to be animal carcinogens.

In short, on the one hand there is a rapidly accumulating data bank on the oncogenic effects of organic extracts of particulates collected from DE (43,44) and, on the other, an absence of such effects (even a possible suppression) in animals breathing whole DE (41). Additionally, in the latter case, an array of nononcogenic effects is observed,

possibly from one or more noncriteria gaseous pollutants in the exhaust stream (41).

Such seemingly contradictory results on the biological impacts of DE illustrate the complexities of the problems facing both health effects researchers and air pollution chemists. For the latter, a high priority goal must be to characterize fully the complex, heterogeneous mixtures of gases, liquid aerosols and fine particulates characteristic of both primary emissions and ambient urban air pollution. Furthermore, as is evident from Figure 1, insofar as possible the "dose" should be characterized at the point of impact on the biological target (i.e., at the site of inhalation). Finally, possible artifacts generated during the collection and analysis of samples should be minimized to ensure that the dose calculated on the basis of chemical analysis and *in vitro* and *in vivo* assays (Fig. 1) is the same as that to which humans or animals are exposed.

In this paper, we shall discuss some recent studies in our laboratories directed toward elucidating the nature and concentrations of certain trace gaseous pollutants and of mutagens present in respirable POM, both in primary exhaust emissions and ambient air. The utilization of new long

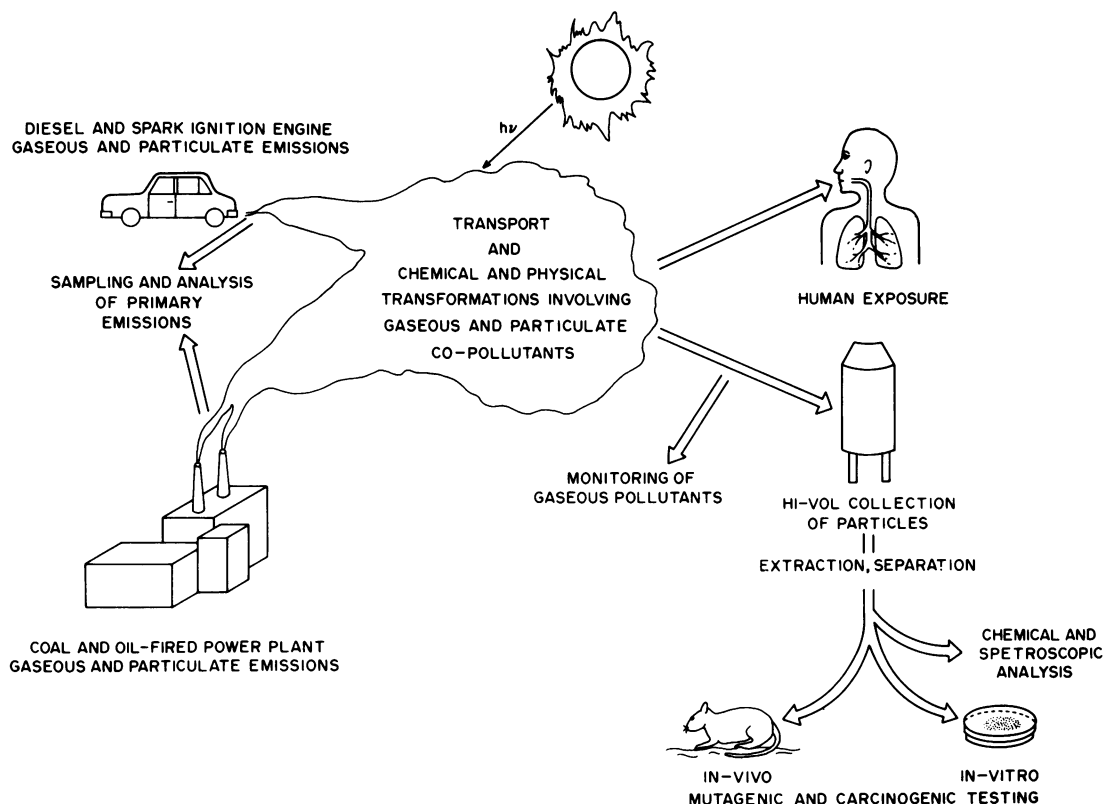


FIGURE 1. Chemical and physical transformations involving transport in the atmosphere and during the sampling of gaseous and particulate copollutants.

pathlength spectroscopic techniques for the gases and integrated chemical/microbiological procedures for identification and quantification of the PAH derivatives in the particulates will be considered. Additionally, some aspects of the rates, products and mechanisms involved with the formation and fate of both gaseous and particulate species in simulated and real polluted atmospheres will be discussed.

Some emphasis is placed on the spectroscopic identification and measurement of gaseous HONO in ambient air and auto exhaust emissions. This highly reactive species is important not only in the chemistry of photochemical oxidant but also contributes to the nighttime (or wintertime) chemistry of polluted urban and suburban atmospheres. Additionally, it poses health concerns as a possible gaseous mutagen and carcinogen (or precursor) or simply as a "trace" toxic pollutant whose health impacts at ambient levels or in exhaust emissions have not yet been well defined.

No attempt will be made to review critically the literature. Trace gaseous pollutants are discussed in the EPA criteria documents for O₃ and NO₂, and particulate atmospheric mutagens and carcinogens have been considered in detail at several recent symposia proceedings (43,45-48) and critiqued in detailed assessment documents (2,5,49).

Gas-Phase Noncriteria Pollutants

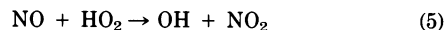
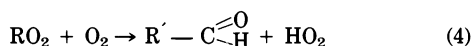
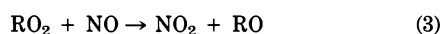
Atmospheric Chemistry

The photooxidation of organics in the presence of NO_x in the natural or polluted troposphere is a highly complex chain reaction. Over the last 25 years, smog chamber studies of simulated atmospheres have shown that the mechanism of O₃ production involves initiation by the attack of hydroxyl radicals (OH) on the organic compounds (RH), followed by a series of well-known atomic and free-radical reactions (51,52). Key steps in its mechanism are shown in Eqs. (1)–(9).

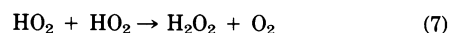
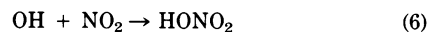
Initiation:



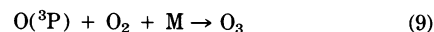
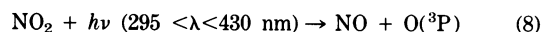
OH Chain Reaction and NO → NO₂ Conversion:



Chain Termination:



O₃ Formation:



Until recently, the attention of most air pollution control officials and health effects researchers has been focused primarily on O₃ formation and NO → NO₂ conversion because of the air quality standards applying to O₃ and NO₂. However, since the late 1950s, smog chamber experiments have also shown, primarily through moderate pathlength (ca. 40-60 m) infrared spectroscopic studies, that the "trace" secondary pollutants shown in Tables 1 and 2 are also formed.

Despite a long-term interest on the part of atmospheric chemists in knowing whether or not nitrogenous species seen in chamber experiments were also present in ambient polluted atmospheres, only peroxyacetyl nitrate was spectroscopically observed in Los Angeles smog (as "compound X" in the late 1950s); until the 1970s, the others were not unequivocally identified, much less quantified in urban air. This was because "conventional" analytical approaches (including those developed for NO_x, O₃ and SO₂) lacked the sensitivity and/or specificity necessary for their unequivocal identifications in the ppb to ppt (part per trillion, one part/10¹²) concentration ranges, especially when they were present in highly complex heterogeneous mixtures.

Long-Path FT-IR Detection of Ambient HONO₂, HCHO and HCOOH

A major breakthrough occurred in the early to mid-1970s when very long pathlength, high resolution infrared spectrometers with rapid scan times were assembled by Hanst and co-workers at the EPA (52,53). They coupled a Fourier transform infrared spectrometer (FT-IR) to a White cell-type multiple reflector system (optical pathlength 417 m) and, in the summer of 1973 in Pasadena, CA, for the first time, unambiguously identified formic acid (HCOOH) in ambient air (52).

In 1976 our SAPRC team set up a modified version of Hanst's instrument (Fig. 2) with an optical pathlength of ca. 1 km for use in field studies in California's South Coast Air Basin (CSCAB); it was used over a 5-year period. During this time, the FT-IR system permitted the first spectroscopic detection of HCHO and HONO₂ in ambient smog and confirmed the presence of HCOOH (53).

Initially, measurements were made at a receptor site, Riverside, CA, ca. 100 km downwind (east) from downtown Los Angeles (DTLA). Subsequently, in the fall of 1978, HCHO and HONO₂, and concurrently HCOOH, PAN and O₃, were determined with this long pathlength FT-IR system during a severe photochemical air pollution episode at Claremont, CA, ca. 60 km east and downwind from DTLA (54).

On October 13, the worst in this 5-day episode (as seen in Fig. 3), the sum of the maximum concentrations of four of these gaseous noncriteria pollutants (PAN = 37 ppb, HONO₂ = 49 ppb, HCOOH = 19 ppb and HCHO = 71 ppb) was ~40% of the O₃ maximum of 0.45 ppm. Clearly, the "dose" experienced by the local population that day could be significantly more damaging to the lungs (and perhaps other targets) than that which might be predicted from research on subjects exposed simply to various concentrations of O₃ in pure air. Exacerbating the health impact on the general population during the episode were levels of respirable sulfate and TSP (which also included high levels of nitrate aerosol)

of ~16 µg/m³ and ~230 µg/m³, respectively, on a 24-hr average.

From the perspective of general health effects, several points should be noted. This was a very serious episode, even for a region known for severe photochemical air pollution, and the maxima shown in Figure 3 for HCHO, HCOOH, HONO₂ and PAN should be viewed as exceptional rather than typical for most urban areas throughout the world. Furthermore, such episodes are not year-round phenomena. Therefore, in the absence of specific industrial or mobile source emissions of these trace species, these maximum values should be viewed as approximate upper limits to ambient concentrations. "Typical" values for light to moderate photochemical air

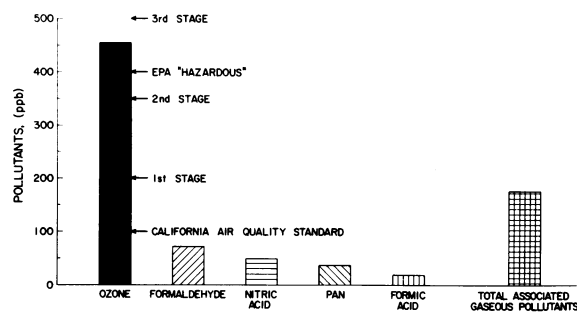


FIGURE 3. Maximum concentrations of ozone and "noncriteria" pollutants determined by kilometer pathlength FT-IR spectroscopy during a severe smog episode in Claremont, California, October 13, 1978.

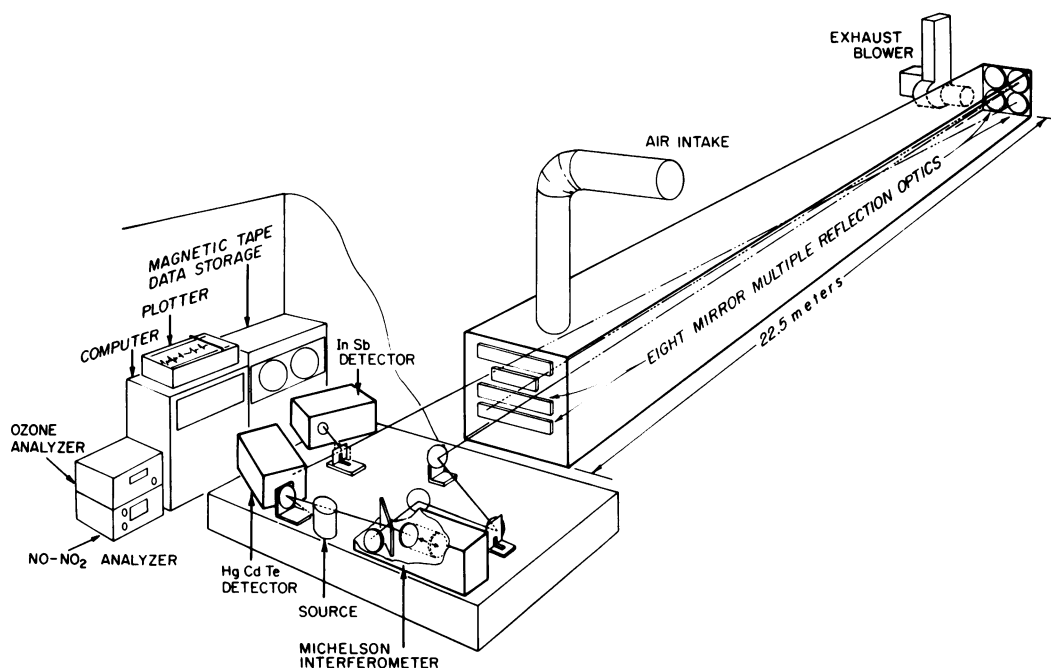


FIGURE 2. Kilometer pathlength multiple reflection infrared cell, and FT-IR spectrometer.

pollution ($O_3 = \text{ca. } 0.08\text{--}0.20 \text{ ppm}$) should be reduced accordingly.

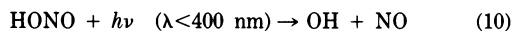
At relatively low ambient levels (compared to occupational exposures) in light to moderate photochemical smog (see Table 1), it seems unlikely that these trace air pollutants pose significant, short-term health hazards to the general population. However, formaldehyde in the ppm range is now generally recognized as a strong carcinogen in animals. Thus it seems prudent to bear in mind that it is present in significant amounts in primary exhaust gases from existing noncatalyst-equipped LDMV and, that if a problem presently exists, it will be exacerbated if there is widespread substitution of MeOH or MeOH-gasoline mixtures for gasoline as a fuel for noncatalyst-equipped LDMV.

The levels of gaseous nitric acid observed during this intense episode (and in less severe, more typical episodes) ultimately may have more impact ecologically in the form of "acid rain" than as a human health problem. Still, it is an irritant and should not be overlooked when considering possible synergistic chemical interactions with its copollutants in photochemical smog. For example, it is a catalyst for the nitration of particulate PAH by NO_2 in simulated atmospheres.

With respect to gaseous formic acid, certainly it is a powerful irritant, but its ambient levels (ca. 1-20 ppb) are quite low. Its possible biological role as a trace air pollutant remains an intriguing question.

Long Path UV/Visible Spectroscopic Detection of Gaseous HONO and NO_3

While use of the kilometer pathlength FT-IR system established the presence and concentration ranges of HCHO, HCOOH, $HONO_2$ and PAN at several sites in southern California, its inherent lack of sensitivity and specificity for gaseous nitrous acid prevented simultaneous determination of this highly important species in atmospheric chemistry. Thus photolysis of HONO in urban airsheds has been postulated to be a prime early morning source of OH radicals [reaction(10)].



As we have seen, these OH radicals initiate chain photooxidations [reactions (1-5)] which lead to $NO \rightarrow NO_2$ conversion and the formation of O_3 , PAN and the noncriteria pollutants shown in Table 1. Indeed, totally arbitrary amounts of "initial" HONO often have been used by modelers as adjustable sources of OH radicals when they set the initial

conditions for kinetic-computer models of ozone formation in ambient atmospheres.

Our interests in detecting and measuring ambient HONO extended beyond its impact on photochemical oxidant production. Our earlier results from outdoor environmental chamber studies (55) showed that in simulated atmospheres containing ambient levels of NO_x (and presumably HONO), secondary amines react rapidly in the dark to form gaseous nitrosamines (though with a rather low yield). Furthermore, in sunlight these are subsequently photooxidized to nitramines (56), at least one of which (dimethylnitramine) is also an animal carcinogen, although weaker than the nitroso derivatives (57,58).

These findings, and earlier observations of an epidemiological nature (59), suggested possible health implications if HONO were indeed to be found in ambient air [as it had been by FT-IR spectroscopic studies of ppm levels of NO_x -air mixtures in environmental chambers (60)]. For example, could the direct inhalation of HONO by animals or humans form nitrosamines *in vivo*, or could it produce other types of health effects?

Ambient HONO. Recently a novel differential optical absorption technique (DOAS) was developed by a German team led by U. Platt and D. Perner at the Institut für Atmosphärische Chemie der Kernforschungsanlage Jülich (61). This long pathlength UV/visible spectrometer has the requisite specificity and sensitivity to identify and measure ambient HONO at concentrations as low as 100-200 ppt. Furthermore, NO_2 , formaldehyde and SO_2 can be measured specifically and concurrently at approximately the same ambient levels. Additionally, O_3 and the gaseous nitrate radical (NO_3) can be determined in another region of the spectrum, ca. 650 nm. Finally, this technique offers the possibility of unambiguous identification and measurement of sub-ppb levels of other trace species having suitable vibronic spectra in the UV-visible region. Limiting factors are their absorption coefficients and the degree of chemical and physical completion of the air parcel studied.

Briefly, the spectrometer (Fig. 4) consists of a broad-band 500 W high-pressure xenon light source, focused and transmitted parallel to the ground in single pass mode through 1-10 km of the atmosphere (61). Selected 40-nm segments of the transmitted intensity are dispersed with a grating monochromator and the wavelength-intensity profiles of these segments are repetitively monitored with a rapid scanning device and a photomultiplier tube. Species with structured absorption spectra present in the light path can be identified, and their concentrations then deduced from differences in the optical densities on and off the key absorption peaks. Absorption

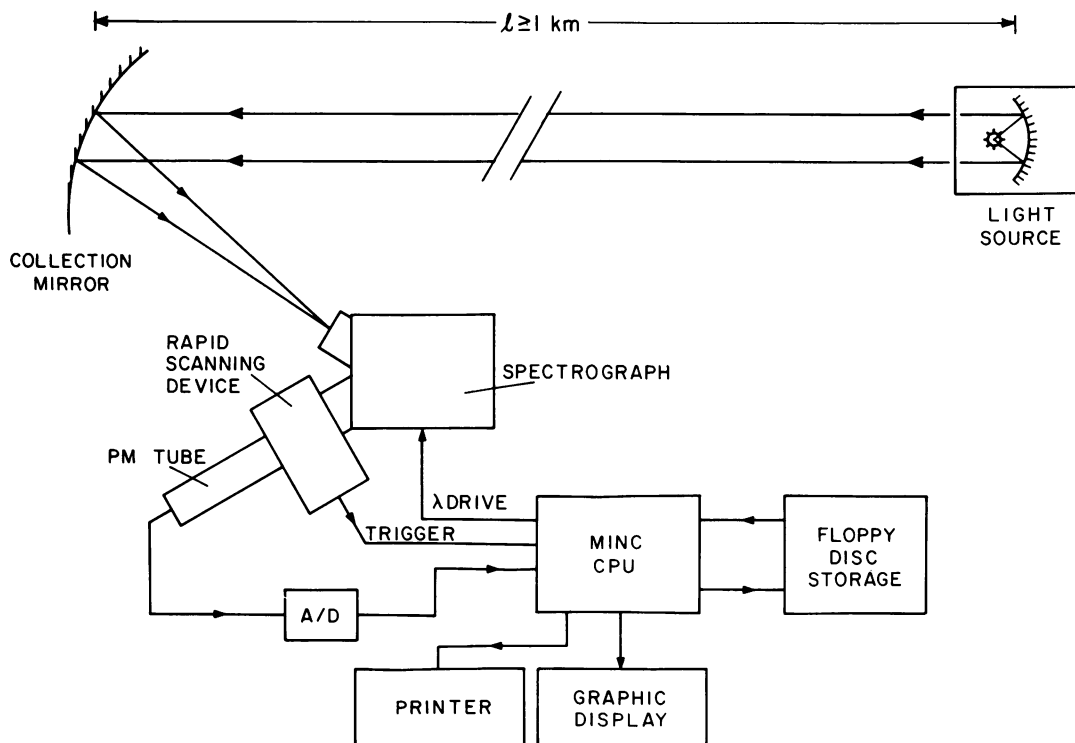


FIGURE 4. Schematic drawing of long path differential optical absorption system (DOAS).

lines with optical densities as low as 10^{-4} (base 10) can be detected. Data accumulation and processing are performed using a DEC MINC-11/23 computer system.

In a collaborative program with Drs. Platt and Perner, using the DOAS spectrometer, we first identified ambient HONO at Riverside and Claremont, CA in the fall of 1979 and, in a series of observations, measured its gradual buildup during the night and its rapid decay after sunrise (62).

Figure 5 shows a series of ambient air spectra taken at our UCR campus from 2109 hours August 4 through 0830 hours August 5, 1979. The optical path was 0.86 km and the beam was 2-20 m above ground. The maximum concentration was 3.3 ppb at 0526 hours PDT. During a series of observations at Riverside and Claremont extending over several days, the highest value of HONO observed was 4.1 ppb; typical values ranged from ~ 1.5 to 2.5 ppb.

During the summer of 1980, in an experiment designed to place the spectrometer at a site characterized by much higher emissions of NO_x from mobile sources, HONO measurements were performed in DTLA near the intersection of two major freeways, CA 7 and U.S. 10 (63). At this site [with the detection system on the campus of California

State University at Los Angeles (CSLA)], two optical paths were used across the freeway (Fig. 6). Path I was 2.26 km long and 50-100 m high; path II was shorter (0.96 km) and closer to the freeway (20-50 m high) but in the same general direction as path I. HONO was again monitored using the absorption lines at 354.1 and 368.1 nm.

Figure 7 shows the time-concentration dependence of HONO_2 on the morning of August 1, 1980. Qualitatively, this time dependence is typical of that for the 25 days on which measurements were taken, the HONO concentration slowly building up to a broad maximum before dawn and then decreasing to levels below our detection limit (~ 0.2 ppb) after sunrise when photolysis occurs (reaction 10). Maximum HONO levels observed ranged from a low of < 0.5 ppb to ~ 8 ppb at 0600 hr (PDT) on August 8, 1980. Most maxima fell in the range 2-6 ppb and were observed between 0300 and 0600 hrs (PDT).

The spectroscopic identification of 1-8 ppb of gaseous nitrous acid for several predawn hours on a number of days at three widely spaced urban/suburban locations in southern California [as well as in Jülich, Germany (64)] has several implications.

The presence of significant levels of gaseous

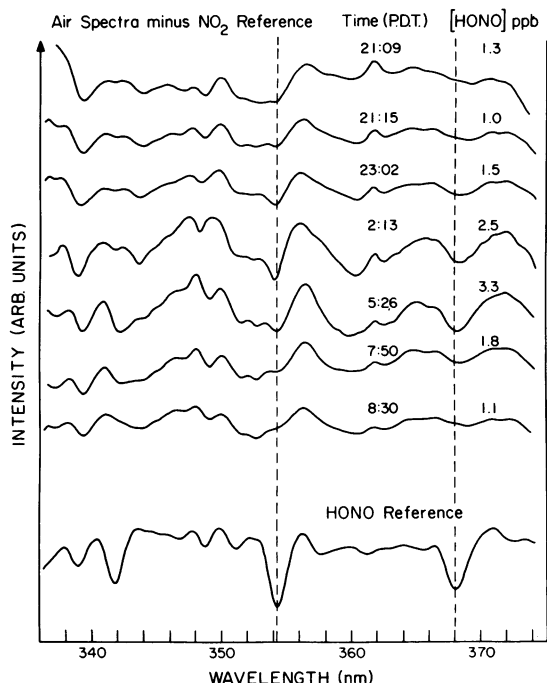


FIGURE 5. Differential UV/vis optical absorption spectra showing the build-up of HONO, Riverside, California, August 4-5, 1979. The bottom trace is a HONO reference spectrum.

ambient HONO in urban atmospheres with high NO_x levels from mobile and stationary sources may be a general phenomenon not restricted to special conditions of photochemical air pollution. However, before this point can be firmly established, many more measurements must be taken over a wide range of NO_x emissions from mobile and stationary sources in several geographically distinct, major polluted urban regions. They should be conducted under a wide range of meteorological conditions, including relative humidity and temperature. Furthermore they should also be conducted in the winter/spring seasons. The characteristic tight radiation inversions and diminished solar radiation in winter might lead to higher ambient concentrations than we have observed in the summer/fall seasons [recall, HONO photodissociates efficiently when exposed to actinic UV radiation, reaction (10)]. Currently we are conducting such wintertime measurements in DTLA.

In terms of atmospheric chemistry, ambient HONO at levels of 3-10 ppb is a key early morning source of OH radicals in DTLA (followed later in the morning by photolysis of HCHO and subsequently of O_3). Furthermore, if these HONO concentrations are included with initial NO_x concentrations, model calculations predict that a greater degree of NO_x

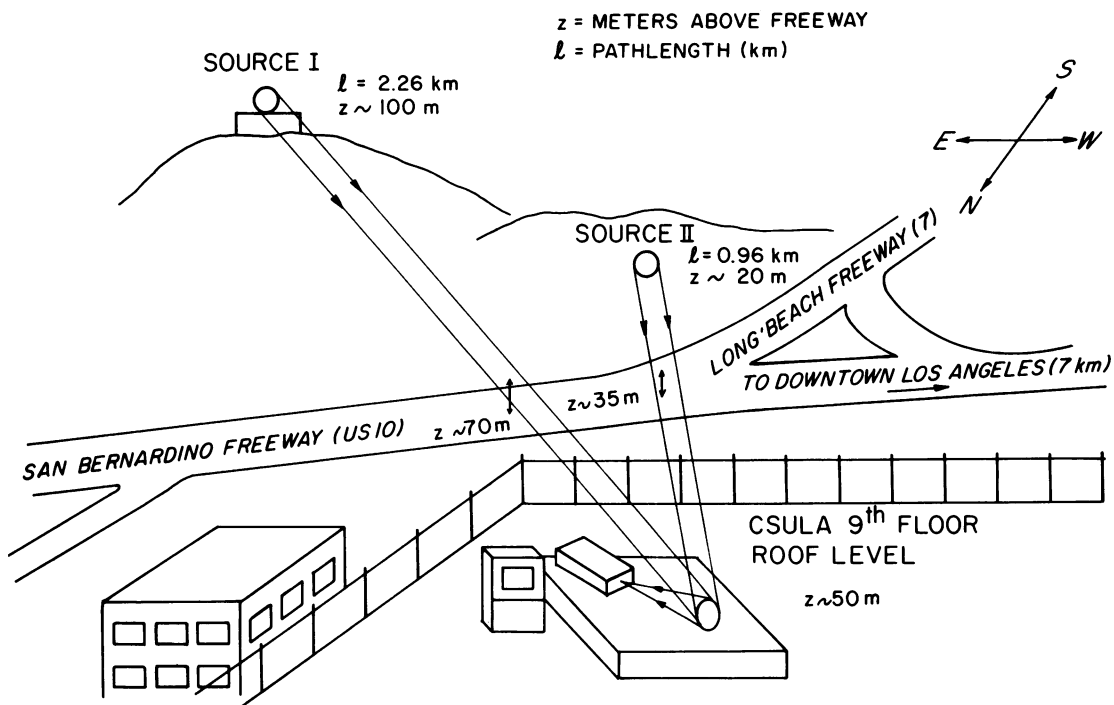


FIGURE 6. Positioning of differential optical absorption spectrometer light source and collection mirror in downtown Los Angeles (California State University campus).

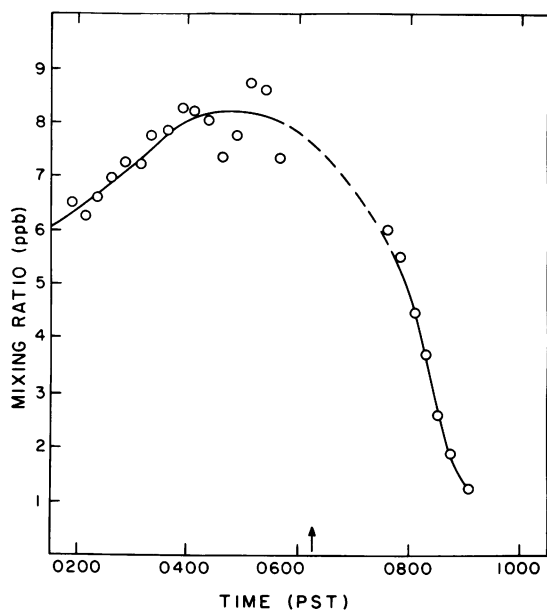
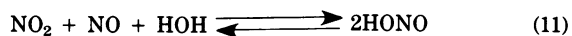


FIGURE 7. Time-concentration profile of HONO monitored by UV/vis long path differential optical absorption spectroscopy in downtown Los Angeles, August 8, 1980 (960 m pathlength).

control will be necessary to achieve given reductions in ozone maxima in "downwind" regions of southern California. Finally, including HONO with initial NO_x levels leads to predictions of accelerated rates of O_3 production which in turn will result in significantly larger calculated O_3 doses. This phenomenon can be viewed as an indirect, but nevertheless real, health effect caused by the presence of ambient HONO (40,63).

Like HONO_2 (26), gaseous HONO may act as an acid catalyst for the nitration of PAH on the surface of POM by NO_2 . The possibility also exists for the direct nitration of these bound PAH by HONO itself (possibly in "solution" on the wet surface of the POM) or by HONO- HONO_2 mixtures, as in laboratory experiments in solution (65). However, the precise nature and source of the nitrating agent might be difficult to establish unequivocally since gaseous HONO exists in equilibrium with NO and NO_2 [reaction (11)].

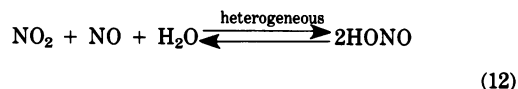


Given the rapid rate with which HONO nitrosates gaseous secondary amines in the atmosphere to form carcinogenic nitrosamines, it would seem prudent to minimize the release of such amines into atmospheres with high NO_x . Since amine levels are generally very low in ambient air, their reaction

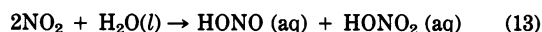
with HONO would not appear to be a threat to general public health. However, the possibility of this reaction should be kept in mind in certain occupationally related situations where higher levels of amines are present in air containing high NO_x levels (26).

The question of possible *in vivo* formation of nitrosamines from inhalation of gaseous HONO, as mentioned earlier, may be more relevant health-wise and should be addressed in some detail by medical researchers. Thus it can be estimated that an average person breathing air containing 4 ppb of HONO ($\sim 8 \mu\text{g}/\text{m}^3$) for 4 hr will inhale $\sim 16 \mu\text{g}$ of the substance. Clearly, the term "trace pollutant" should be used with care when describing the ambient levels of possible carcinogens, or precursors to carcinogens, such as HONO.

Sources of HONO; Short Path Spectroscopic Studies. To date the sources of the ambient HONO are not known unequivocally. Heterogeneous processes appear to be involved because the known nighttime homogenous chemistry is too slow to account for the concentrations we observed. One possibility is the process (12),



while another is the disproportionation of NO_2 in aqueous aerosols [Eq. (13)].



The latter involves the conversion of NO_2 to nitric acid and hence represents a further sink for NO_x and a production mechanism for nitrate ion (66,67).

Currently, there are arguments pro and con for both of these processes (as well as other reactions); resolution of the problem awaits the results of further research, some of which is now underway and will be described below.

Proceeding with the hypothesis that heterogeneous processes (one or more) forming HONO may occur immediately after primary emissions of NO_x (e.g., from LDMV or diesel trucks) are injected into the atmosphere, we developed a short-path (3.57 m) DOAS system suitable for monitoring primary emissions (67-69). It can be used for the spectroscopic detection and measurement of HONO and NO_2 concurrently at levels as low as 50 ppb. Additionally, concentrations of $\text{HCHO} \geq 500$ ppb can be measured when NO_2 levels are relatively low (≤ 1.5 ppm). This reduced sensitivity results from certain HCHO features being overlapped by NO_2 in this spectral region.

In 1980-81, the short path spectrometer was

developed, tested and interfaced to the dynamometer and constant volume sampling (CVS) facility (Fig. 8) at the California Air Resources Board (CARB) Haagen-Smit Laboratory in El Monte, California. Initial investigations of emissions of HONO, NO₂ and HCHO were conducted for a range of vehicles (a total of 15 cars and pickup trucks) and engine operating conditions. The results of these initial studies can be summarized as follows (67-69).

HONO was identified for the first time as a component of the contents of sample bags filled with LDMV exhaust according to standard procedures (point B, Fig. 8) and of the effluent from the CARB-CVS apparatus (point C, Fig. 8).

A sample bag filled with a mixture of air (dilution factor of 5.12) and exhaust gases from a 1980, six-cylinder, 300-cubic inch pickup (American-made) with high NO_x emissions contained 8.5 ppm HONO and 67 ppm NO₂ (Table 3) when the vehicle was operated in a standard highway driving cycle. A CARB chemiluminescence analysis of the same sample gave [total NO_x - NO] = 69 ppm, in good agreement with the spectroscopic analysis. HONO comprised ca. 4% of the NO_x in the bag and was present at ca. 13% of the NO₂ concentration.

Highly variable amounts of HONO and NO₂ and highly variable ratios of these quantities were observed in the effluents from the ARB-CVS system (point C), depending on the test vehicle and the particular operating conditions (i.e., highway cycle, cold CVS II or loaded modes, and at high speeds, low speeds or idle). Over the range of makes, model years and mileages, and the various simulated driving conditions, HONO concentrations at point C went from virtually nondetectable (≤ 50 ppb; NO₂ ca. 90 ppb) to a maximum of ~ 2.59 ppm (NO₂ = 29.9

Table 3. 1980 U.S. pickup, six-cylinder, 300 cu. in. operated at standard highway driving cycle (CARB)^a

Parameter	Concentration
NO _x analysis of sample bag contents ^b	
HONO	8.5 ppm (DOAS) ^c
NO ₂	67 ppm (DOAS) ^c
Total NO _x	210 ppm (chemiluminescence)
NO	141 ppm (chemiluminescence)
(NO _x - NO):	69 ppm
HONO/NO ₂	0.13
HONO/NO _x	0.04

^aMileage = 9381.

^bBad dilution factor: 5.2; T = 24.4°C, RH = 48.2%.

^cDifferential UV/visible absorption spectrometer (62,68).

ppm) when the 1980 pickup cited in Table 3 was run at high speeds on a loaded operating mode.

For all seven vehicles operated in the cold CVS II mode, larger absolute concentrations of HONO (and higher ratios of HONO/NO₂) were observed in the first phase of this test (cold start, 506 sec cruise) than in phases II and III (862 sec cruise and warm start and 505 sec cruise after a 10-min soak, respectively). HONO/NO₂ ratios ranged from 0.20 to 0.40 in phase I to 0.07 to 0.20 in phases II and III.

While these preliminary results are interesting, we stress that in these initial studies both absolute and relative HONO and NO₂ concentrations in the effluent from the LDMV tested on the CARB-CVS system were highly variable and depended on the test vehicle and operating mode. Furthermore, the mechanism(s) for HONO formation in this exhaust train system is not yet clear. It may be primarily from heterogeneous physical (e.g., wall adsorption) and chemical (e.g., NO₂ hydrolysis) processes. These could occur upon dilution of the hot exhaust gases with air and subsequent cooling during passage of the exhaust gases through the sampling train and into the "standard" exhaust sampling bag (Fig. 8, point B) or out the blower (Fig. 8, point C).

In order to determine whether the observed HONO was formed entirely on the walls of the CVS dilution-sampling-blower system and not actually present in the hot gases at the exit of the car's muffler/tailpipe (i.e., Fig. 8, point A), we recently designed and developed a novel shortpath (31.2 m) DOAS system (69) with optics open to the atmosphere. When this instrument was placed in the open atmosphere approximately 3 m from tailpipes of a series of LDMV run on a CARB chassis dynamometer at the El Monte, CA laboratories, we found that at least certain "high" NO_x vehicles emit HONO directly as a primary pollutant. Additionally, benzaldehyde and SO₂ were found for a 1977 LDMV run on gasohol containing approximately 10-20%

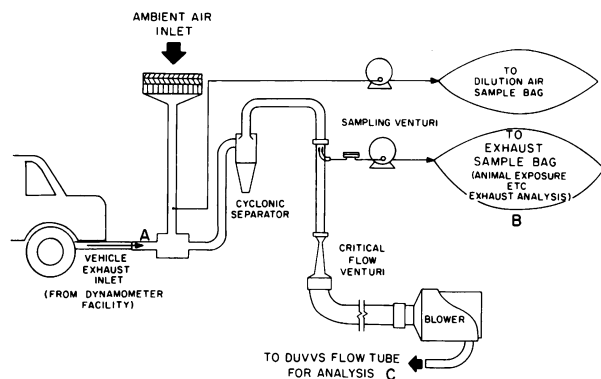


FIGURE 8. Vehicle exhaust constant volume sampling system at the El Monte Air Resources Board laboratory adapted to obtain short path UV/vis differential optical absorption spectra at points A, B and C.

ethyl alcohol. The observation of directly emitted HONO is also consistent with our results obtained from a recent 0.5 km pathlength DOAS experiment (69) in which the diurnal profiles of HONO, HCHO and NO₂ were measured for a number of days adjacent to a major Los Angeles freeway and immediately adjacent to a residential area. The observed diurnal variation in HONO levels strongly suggested that HONO was either being directly emitted or very rapidly formed from freshly exhausted NO_x.

Given this major caveat, however, our short-path DOAS results in Table 3, showing over 8 ppm of HONO in the sampling bag (Fig. 8, point B) containing diluted exhaust from an LDMV having large NO_x emissions, raise several relevant chemical and biological issues. For example, if the contents of the sample bag were exposed to room light even for a short period before product analysis (e.g., for aldehydes), the photolysis of HONO to produce OH radicals could lead to secondary reactions significantly modifying the composition of the original exhaust sample.

Furthermore, exposure of animals to diluted whole exhaust from diesel (41) and spark ignition engines for short- and long-term periods is a very important type of health effects research. However, one must now explore the implications of such experiments if these animals, in addition to NO₂, NO, CO and the other known gaseous and particulate copollutants, are also being exposed to significant, but unrecognized, levels of HONO. This question warrants investigation, in part because of the observations of Pepelko on whole diesel exhaust cited earlier (41).

Measuring HONO levels in exposure chambers currently being used for either animals or humans is clearly useful, and we plan to conduct such studies during 1982. Depending on the results, one might also consider the possible need for health effects studies of animals exposed to synthetic atmospheres containing known amounts of HONO (and associated NO and NO₂) in air.

The Nitrate Radical, NO₃. As is the case with HONO, this trace nitrogenous species, the product of the reaction of O₃ with NO₂, has been known for years in smog chamber systems. It was not until 1979 that it was identified in polluted urban/suburban atmospheres by the SAPRC/Jülich team using the long path DOAS instrument at several locations in southern California (70) and recently in cleaner air in Germany and the California desert (71).

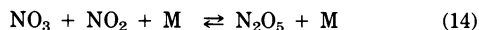
NO₃ was monitored during the nighttime via its strong absorptions at 623 and 662 nm; the minimum detectable optical density corresponded to 5.6 ppt NO₃ for the latter band at a pathlength of 970 m. It photodissociates readily in sunlight, and ambient

concentrations drop below the minimum detection level.

Figure 9 shows the concentration-time profile for NO₃ on the evening of September 12, 1979 at Riverside. Measurements on 15 days in August and September 1979 gave peak concentrations which varied from below our detection limit up to 355 ppt at 1900 hrs (PDT) on September 18 (70).

Just what direct health effects such small ambient concentrations of NO₃ (5-350 parts/10¹²) may produce are not known; quite possibly they are negligible. However, the nitrate radical is a very labile species, reacting with cresols and olefins at such a fast rate that, even in moderately polluted air, consumption of these organics by reaction with NO₃ appears to dominate over the OH radical attack previously believed to be their major sink (72). Thus the possibility of ambient NO₃ abstracting H atoms from organics to form nitric acid and free radicals (e.g., the phenoxy radical from cresol) or directly nitrating certain PAH in ambient POM should not be overlooked. Furthermore, the possibility of gaseous nitrate free radical acting as a possible mutagen and/or carcinogen should not be dismissed out of hand.

In one major respect, chemically, it is an important species. Thus NO₃ is included in all models of the odd nitrogen chemistry of the atmosphere. It is in equilibrium with N₂O₅ through reaction (14).



The N₂O₅ may be hydrolyzed subsequently to yield nitric acid.

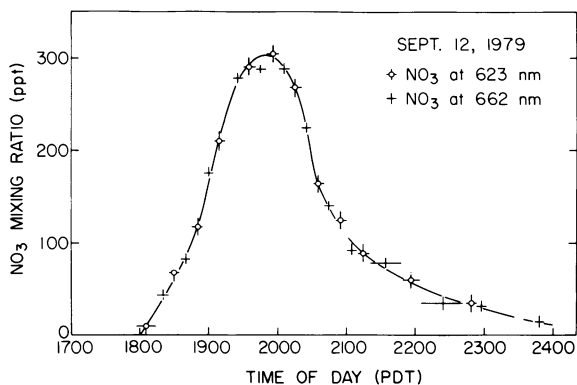
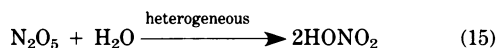


FIGURE 9. Time-concentration profile of NO₃ monitored by long path differential optical absorption spectroscopy in Riverside, California, September 12, 1979 (970 m pathlength).

Reaction (15) is likely to proceed heterogeneously in the atmosphere and thus may be a nighttime source of HONO_2 which, along with the major daytime process [reaction (6)], may contribute to the decrease in the pH of precipitation associated with NO_x pollution. Our recent measurements of NO_3 concentrations as a function of relative humidity at four desert sites in California (71) using a DOAS system over pathlengths of 3–17 km support this latter hypothesis.

Chemical Mutagens in Combustion-Related POM

Central to the validity of risk-benefit assessments of combustion-generated POM is a reliable data base that can be used to calculate, with a reasonable degree of certainty, the chemical natures and concentration levels of the “doses” to which various segments of the population are (or will be) exposed. Unfortunately, the chemical data bank for primary (direct emissions) and secondary POM, while improving rapidly, still suffers from major gaps and contradictions. In order to make a substantial improvement, one must determine the sources of directly mutagenic ambient POM, as well as the levels to which urban/suburban populations may be exposed. One must also unequivocally identify, and subsequently quantify, those chemical species which are direct mutagens present in ambient fine particulates as well as in primary emissions of POM from mobile and stationary sources, and relate them, if possible, to the phenomenon of “excess carcinogenicity.”

The chemical transformations (and their associated biological implications) that occur when the organics (especially PAH) on surfaces characteristic of different types of primary POM react during the daylight and nighttime hours must be determined. Such processes include not only PAH reactions (1) with oxygen in clean air, (2) with the major gaseous pollutants (NO_2 , O_3 and SO_2) and (3) with their noncriteria copollutants (e.g., HONO , HONO_2 , NO_3 and PAN), but also (4) with such labile species as singlet molecular oxygen [$\text{O}_2(^1\Delta)$] and the hydroxyl and hydroperoxyl radicals (26,73,74).

It is also necessary to account for the fact that the chemical and biological properties of both primary POM (as collected, for example, directly from the exhaust of a diesel engine or the plume from a coal-fired power plant) and secondary POM (as collected from urban atmospheres) may be substantially modified chemically by “artifacts” generated during the sampling and analysis procedures (75).

Developing such a comprehensive data base, given the chemical and physical complexity of POM, is

well recognized as a formidable task. However, since the Ames bacterial assay (76,77) was first applied to the detection and chemical characterization of ambient particulate mutagens in the period 1975–1977, research in all of these areas has expanded and accelerated at an astonishing rate.

It is beyond the scope of this paper to review and critique this rapidly expanding mass of interdisciplinary information. [See instead the earlier review/assessment (74) and the comprehensive treatment delivered at this Symposium (78).] Instead, I shall discuss recent research results (some unpublished or in press) from our integrated chemical/microbiological studies focusing on (a) thermal reactions of PAH with NO_2 , and (b) PAH oxidation by sunlight and ozone. Brief reference will be made to the roles of other reactive intermediates such as $\text{O}_2(^1\Delta)$ and the OH and NO_3 radicals, all of which are involved in photochemical air pollution, a summertime phenomenon in the northern latitudes. PAH do not seem to react with SO_2 at ambient levels (79), so SO_x -PAH-air chemistry will not be considered here [note however SO_3 may react with PAH (79)].

As with the gaseous noncriteria pollutants, the perspective remains that of an atmospheric chemist dealing with the chemistry of the “dose.” Thus in our laboratories, the Ames test is employed as a highly sensitive, bacterial assay which has proven invaluable for the rapid detection of chemical mutagens in complex environmental mixtures. Additionally, along with chemical/physical separation and spectroscopic techniques, we use the assay in the isolation, purification and proof of structure of these mutagens. It is not used to infer possible carcinogenic potencies of the chemical mutagens themselves, nor of the spectrum of POM samples studied.

In this context, several years ago it became clear that to establish correlations between the mutagenicities of ambient POM samples with other indicators of air quality (e.g., NO_2 or O_3 levels), or to determine quantitatively the relative mutagenic activities of a series of HPLC fractions of an organic POM extract, it was necessary to improve the precision and accuracy of our intra-laboratory microbiological procedures. Therefore, factors determining the precision and variability of the Ames test were investigated (80).

The most important source of variability in the agar-overlay method as used in our laboratory proved to be nonuniformity in the soft agar layer thickness. This problem was solved by use of an agar-leveling table we developed for this specific purpose. Several other procedural elements also contributed to improved precision. These included temperature uniformity during incubation, incubation interval, consistency of plate agar volume, completeness of mixing

the soft agar overlay, improved methods of storage for tester strains and expanded use of several high purity mutagens as internal standards. When the variables were well controlled, the precision of our test results for assays carried out on a given day improved from ± 200 – 300% to $\sim \pm 10\%$.

As an example of the current, overall intralaboratory precision attainable for the direct mutagenicities of ambient POM samples, Figure 10 shows dose-response data for four "identical" samples collected concurrently, but independently, on our SAPRC "mega-sampler" [a large-scale high volume scripter (hi-vol)]. It should be stressed, however, that these are intralaboratory comparisons of POM samples collected simultaneously, subsequently extracted on the same day and finally tested concurrently. Significantly more variability would have been seen if this were not the case, especially in the assays employing microsomal activation, as the potency of various S9 preparations varies widely with age, source, etc.

Diurnal Variations in the Mutagenicity of Ambient POM

In order to obtain sufficient sample for analyses, most studies to date have determined the mutagenicity of ambient POM from hi-vol instruments run for periods of 24 hr or longer (recently in some cases this has been reduced to 12 hr). However, even collection periods of 12 hr can average out any short-term maxima and minima in, for example, mutagen densities (revertants/ m^3 of sampled air). Furthermore, such 12–24 hr (or longer) data may lack sufficient time-resolution to permit wholly reliable assessments of the nature of the mutagen sources (i.e., mobile vs. stationary source emissions, or primary vs. secondary pollutants).

Therefore, in 1980–1981 we conducted investigations of the diurnal variations in the mutagenicity of ambient particles collected simultaneously at the same three widely spaced sites in southern California used for our long-path spectroscopic studies [i.e., at CSLA at the intersection of several major freeways near DTLA and at the two downwind stations, Claremont and Riverside (81,82)].

Collections were made every 3 hr for a 24-hr period from 0–2400 hours (PDT) on September 12 and 17, 1980 and from 1200–1200 hours (PDT) on March 11–12, 1981. Inlet ports were 30 m, 15 m and 5 m above ground at DTLA, Claremont and UCR, respectively. At each sampling site, five standard hi-vol samplers each operating at 40 SCFM ran 3 hr between changes of the Teflon-coated filters; a sixth sampler at each site operated for the entire 24 hr without filter replacement. This gave an experi-

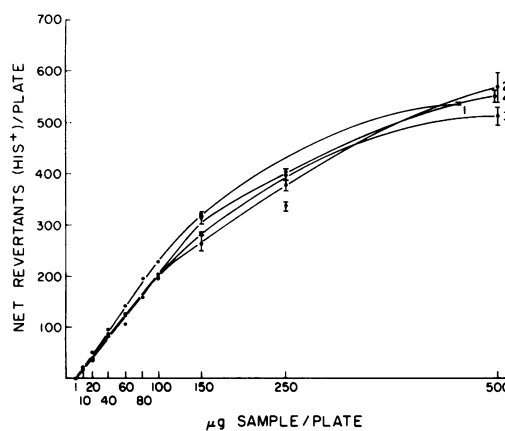


FIGURE 10. Mutagenic activity of four samples of ambient airborne particulate material collected concurrently in El Monte, California, on October 2–3, 1979 (collection time, 27 hr and 18 min; filter type, Teflon-impregnated glass fiber; sampled volume, 7420 m^3 of ambient air). Dose-response curves on strain TA 98 without metabolic activation.

mental 24-hr average sample which could be compared to the 24-hr averages calculated from the sum of the 3-hr averages.

The results at three sites across southern California, and at two different seasons, can be summarized as follows:

- "Time resolved" ambient particulate mutagenicities (Fig. 11a) display to a significant degree the characteristics of a primary pollutant. Thus the 3-hr average mutagen densities relate well to both vehicular emission rates and to atmospheric mixing ratios. The latter is shown by the high correlations of mutagen densities with CO , NO_x and lead levels (Fig. 11b).

- Short-term peak mutagen densities can be much higher than 24-hr average values commonly reported in the literature. This is relevant to health risk-assessment calculations. Thus, for example, one must consider peak doses, as well as 24-hr or longer average doses, when evaluating the probable mutagenic impact of ambient particulate matter on the general population.

- Nitroarenes may contribute substantially to the mutagenicity of ambient POM in southern California [as they apparently do in Fort Wayne, Indiana (83) and Durham, North Carolina (28)]. This is seen by the significantly reduced response (50–60% compared to TA 98) to the Rosenkranz nitroreductase-deficient strain TA 98NR (84,85) for all samples collected in the September 24-hr period. Furthermore, the activities with TA 98NR showed a diurnal variation similar to that for TA 98.

- The good agreement between the activities of

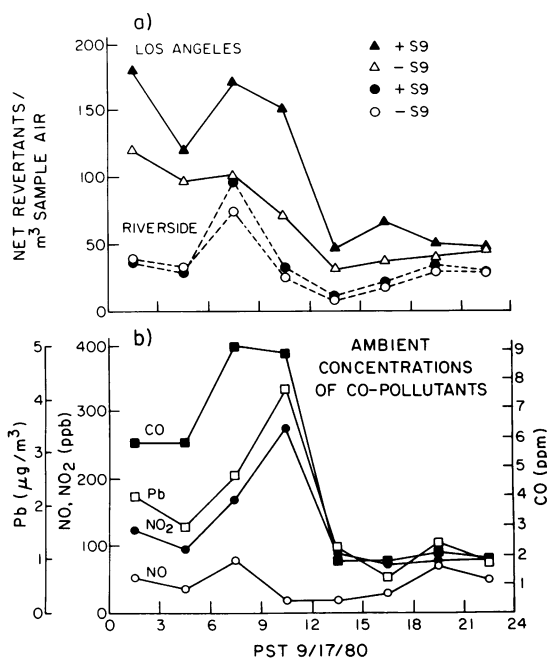


FIGURE 11. Plots of (a) diurnal variations in mutagen densities (rev/m³) of ambient POM collected in Los Angeles and Riverside, California and (b) ambient concentrations of copollutants at Los Angeles collection site September 17, 1980.

the experimental 24-hr averages calculated from the corresponding eight 3-hr samples suggests that chemical transformations of mutagenic material collected on the filters are either very fast or very slow relative to the time scale used in these experiments.

- The increased contribution of promutagenic activity to the mutagenicity of the particles during periods of high emission rates (Fig. 11a) and its subsequent return to lower levels relative to the direct activity suggest that some promutagenic materials present in freshly emitted POM are subject to rapid destruction in the atmosphere.

Formation and Fate of Mutagens in POM: Nitroarenes

In the case of ambient POM and diesel exhaust particulates, only after a significant biological impact was reported (i.e., mutagenicity in the Ames bacterial assay) did extensive efforts begin among chemists to analyze extracts of various types of POM for directly mutagenic PAH derivatives and to inquire in depth as to their environmental sources and sinks.

The analytical problems of separating, identifying and quantifying the species responsible for the

direct mutagenicities of the complex mixtures characteristic of combustion-related POM have proven formidable indeed. Typical of these problems is the fact that mere traces of certain compounds with ultra-high activities in the Ames assay (e.g., dinitropyrenes) can make significant contributions to the overall mutagenicity of the crude extracts. Other analytical difficulties arise from the relatively low vapor pressures of certain polar oxygenates which, coupled with their ease of thermal decomposition, lead to difficulties in introducing samples to conventional GC/MS instruments. However, significant progress in dealing with sampling and analytical difficulties has been made.

Diesel POM. Over the last two years intensive studies in several laboratories have shown that a number of nitro-PAH are present in substantial amounts in diesel POM (86,87) and that at least one of these, 1-nitropyrene (1-NO₂-P), is a strong bacterial mutagen with and without S9 activation (85,88,89). Recently, other mononitro-PAH, as well as several dinitropyrenes, have been tentatively identified, most of them by GC/MS and MS/MS techniques and fluorescence spectroscopy; some of these may also prove to be strong direct mutagens [see reports from the 1981 EPA Diesel Symposium (43)].

Table 4 lists three nitroarenes we have recently isolated from extracts of diesel POM, purified and characterized by GC/MS and several spectroscopic techniques (90); 1-NO₂-P [confirming earlier reports (86,87)], 9-nitroanthracene [(9-NO₂-A), previously tentatively identified (86)], and 6-nitrobenzo(a)pyrene [(6-NO₂-BaP), not previously reported]. Also listed is a new type of direct mutagen in diesel POM. It is a K-region lactone related to pyrene (4-oxapyrene-5-one, I, see Fig. 14). Although only a weakly active promutagen (9.3 rev/μg, 20% S9, TA 100), we believe that this lactone may be representative of a large class of PAH transformation products, for example, in ambient particulate matter.

Nitroarenes also have been found (or tentatively identified) in POM from ambient air (91), carbon black used in toners (88,92) and typewriter ribbons (93) and in simulated atmospheres (26,75). Indeed they seem to be ubiquitous in modern society. Clearly this class of compounds warrants the increased attention being shown by atmospheric scientists and biomedical researchers.

For example, it is essential that carefully designed and executed animal exposure studies with potentially harmful nitroarenes be planned and initiated on a high priority basis (some are no doubt well underway). First priority should go to those nitro-PAH known to be in POM and which show significant activities in short-term bioassays. Candidate com-

Table 4. Activities on strains TA 98 and TA 98NR of highly purified samples of some components of diesel particulate extracts, "standard" mutagens and several other mutagens of environmental interest.

Compound	Specific activity on two Salmonella strains, rev/μg			
	TA 98		TA 98NR	
	-S9	+ S9 ^a	-S9	+ S9 ^a
Diesel POM components				
1-Nitropyrene	3500	6100	380	3700
6-Nitrobenzo(a)pyrene	0 ^b	1500	0 ^b	1800
9-Nitroanthracene	0.30	0.32	0.14	0.13
4-Oxapyrene-5-one (I)	9.3 ^c	3.7	n.d. ^d	n.d. ^d
Standard mutagens				
2-Nitrofluorene	417	340	44	230
Quercetin	11	16	12	18
Benzo(a)pyrene ^e	0	700	0	710
Other mutagens				
(1 or 3)-Nitrobenzo(a)pyrene ^f	5300	3400	1100	3800
(3 or 1)-Nitrobenzo(a)pyrene	3600	3700	790	3600
Perylene	0	0 ^g	0	0.8
3-Nitroperylene	15	12000	7.7	9800

^a2% (v/v) mix.^bResponse at highest dose tested was less than twice spontaneous reversion frequency.^c20% (v/v) mix, TA 100.^dNot determined.^eAlso present in diesel POM.^f1 and 3 isomers separated but not as yet characterized.^gSome activity is seen at much higher concentrations of S9.

pounds might also include several whose "tentative" identifications in POM seem reasonable, and/or nitroarenes whose actual (or predicted) rates of formation from PAH and NO₂ in primary exhaust POM and/or secondary ambient particulates are relatively fast.

Sample Purity. Before embarking on such expensive and time-consuming studies, however, it is essential to ensure that ultra-high purity nitroarenes are available, and that detailed precautions are taken to maintain the chemical integrity of the "standard" nitro-PAH samples administered during the entire period of the animal studies.

With respect to the purity issue, our studies over the past 5 years of three mononitro isomers of BaP have shown (not surprisingly) that their mutagenicities are strongly dependent upon the position of substitution in the ring. This has led to major contradictions in the literature concerning the direct mutagenicity of 6-NO₂-BaP. Thus it has previously been described both as a "strong" (32) and a weak-moderate direct mutagen (25,94). Actually, while 6-NO₂-BaP is a stronger promutagen with strain TA 98 than BaP itself, after careful purification (HPLC, silica gel, 15% CH₂Cl₂ in hexane, UV detector "off"), it is not directly active to this Ames strain at doses up to 50 μg/plate (90).

The reason for the wide range of activities previously reported for this compound can be inferred

from examination of Table 4. This shows the direct and activatable mutagenicities (S9, 2% v/v mix) of highly purified samples of 6-NO₂-BaP and the corresponding 1-(or 3) and 3-(or 1) isomers (as well as of 1-NO₂-P and 9-NO₂-A). The 1- and 3- isomers have direct activities > 3000 rev/μg for strain TA 98. Thus it seems plausible that the direct mutagenicities cited earlier for 6-NO₂-BaP were probably due to contamination with small amounts of the 1- and 3- isomers (90).

Similar considerations apply to earlier reports on the direct mutagenicity of 1-NO₂-P, except that in this case contamination is probably by small amounts of dinitro derivatives. These, as noted above, are extremely powerful bacterial mutagens (88,89) and likely responsible for part of the direct mutagenicity observed for diesel particulates, carbon black toner and possibly other types of POM. Thus the direct activity of our purified sample of 1-NO₂-P (Table 4; 3500 rev/μg with TA 98) was a factor of 3 or 4 less than the earlier published values. Happily, recent interlaboratory comparisons of direct activities on highly purified samples of 1-NO₂-P are now in good agreement. Induced activities vary over a wider range, which is to be expected in view of the well known effects of the source and dose of the S9 mix employed for microsomal activation (95).

In short, this short-term *in vitro* assay system for environmentally important nitroarenes is very

sensitive to traces of isomeric impurities. In view of this, prudence suggests extreme care be given to purifying these compounds prior to testing them as possible animal carcinogens.

Reactivities of PAH to Nitration. Since the first demonstration of the facile formation of nitroarenes from PAH deposited in filters and exposed to low levels of NO_2 in air (25,26,75), there has been increasing attention to the general phenomenon of nitration of PAH. While the basic chemistry *per se* is interesting, the primary impetus has been the observations we have been discussing (i.e., certain nitroarenes are powerful bacterial mutagens and widely distributed in the environment).

Possible mechanisms of atmospheric formation, lifetimes and environmental fates already have been discussed in some detail (26,65,74,78) and thus will not be discussed extensively here; some aspects however seem worth brief comments.

In the initial studies of the nitration of PAH deposited on glass fiber filters by NO_2 in air, it was found that the reaction occurred in the dark at NO_2 levels as low as 0.25 ppm in air [the state of California air quality standard for NO_2 (25,26,75)]. The reaction was acid-catalyzed and had yields of ~50% for exposures of BaP and perylene for several hours. Interestingly, its rate depended upon the structure of the PAH in accordance with the theoretical considerations of Dewar on aromatic nitrations in solution (96,97). Thus the experimental rates of nitration with NO_2 were BaP and perylene > pyrene > chrysene; the theoretical relative rates are BaP (6-position) = 31, perylene = 22, pyrene = 4.9, chrysene = 1 (26). Additionally, it was found that the distribution of isomeric mononitro-BaP derivatives generally followed the electron densities calculated for various positions in the molecule in that the major product was the 6- NO_2 isomer with smaller yields of the 1- and 3- isomers.

Recent experiments dealing with the nitration of PAH present on the surfaces of actual POM suggest generally similar phenomena occur (although perhaps with different mechanisms). Thus the rates of loss of PAH in soot exposed to NO_2 /air mixtures also followed Dewar's theory (98). Furthermore, recent analyses of urban particulate matter collected from air "doped" with ca. 1 ppm added NO_2 showed similar PAH degradations [i.e., BaP ~ 60%, benz(a)anthracene ~ 40% and pyrene ~ 20% (99)]. Exposure of the ambient POM to the excess NO_2 also caused an increase in the mutagenicity but did not change the affinity to the TCDD-receptor protein (95). Interestingly, in similar experiments with O_3 added, no such degradation was seen. Certainly, as the authors suggest, this approach seems useful for studies of artifact formation when sampling ambient POM.

A recent comprehensive treatment of the rates and mechanisms of the nitration of PAH in solution (but under environmentally relevant conditions) is that of Neilsen (65). He investigated the decomposition rates of 21 PAH and nine substituted anthracenes in dilute solutions of nitric and nitrous acids, established a reactivity scale and correlated the results with several other parameters (as well as with other possible atmospheric processes). Finally, based on the earlier treatment of Clar (100), he developed a simple and useful approach to estimating the reactivity of PAHs in such reactions.

Currently in our laboratory, a number of PAH are being deposited on lampblack and exposed to sub-ppm levels of NO_2 in air. Experiments are being carried out in the dark and in the light and with and without a nylon prefilter to remove catalytic traces of HNO_3 present in standard tanks of dilute NO_2 in air. Among the most interesting results is that, in addition to the expected 3- NO_2 isomer (26), at least one, and possibly two, other nitro isomers of perylene are formed (101,102). Furthermore, they have significantly different direct and activatable mutagenicities on strain TA 98. Additionally, the 3- NO_2 isomer has a dose-response curve strikingly different from previously tested nitroarenes (101,103).

From a chemical standpoint, perhaps the most interesting result of our initial studies is that the rate of nitration of the perylene appears to go up dramatically with concurrent exposure to actinic light. If this proves to be true, the implications to fundamental photochemistry of this simulated atmospheric system, as well as to the environmental formation and fate of this PAH (and possibly others present in ambient POM), are intriguing.

In short, the mechanisms by which nitroarenes are formed from pure PAH deposited on various filter surfaces (e.g., glass or Teflon), and/or real POM deposited on various substrates such as diesel soot or fly ash from coal combustion (104), are not presently known. Depending on the circumstances, they may involve free radical processes and/or, on the wet surfaces, radical cations (78). Certainly (as we have discussed earlier), in addition to NO_2 , ambient polluted air contains nitric and nitrous acids which can act as catalysts; indeed they and the NO_3 radical also might participate directly as nitrating agents in the complex gas/wet surface substrate systems characteristic of ambient POM. This seems to be a research area worthy of long-term, fundamental studies.

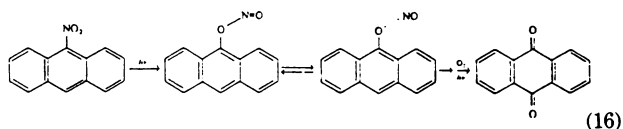
It is worth noting parenthetically that, when considering radical reactions, the possibility of nitration of volatile aromatics such as benzene and toluene in the atmosphere (38) or in heated stack and exhaust gas plumes should not be overlooked (65,78). Thus in California there is widespread sub-

stitution of toluene and other light aromatics (as high as 35-40% of the fuel) in place of lead alkyls to maintain the octane rating of unleaded gasoline used in catalyst-equipped vehicles. Furthermore, while the yields of simple nitroaromatics (including gaseous and particulate nitrophenols and cresols) are probably very low under ambient conditions (105-107) [ring opening is a major process in OH attack on benzene and toluene in irradiated NO_x -air mixtures (108)], they may include some compounds that are mutagenic. Interestingly, one postulated mechanism for the formation of nitrophenols or nitrocresols involves phenoxy-type radicals (108); similar radicals may also be involved in the nitration of polycyclic aromatic compounds.

Photodecomposition. One probable environmental fate of certain nitroarenes is their photodecomposition, ultimately into quinones, and possibly phenolic derivatives. Thus on irradiation, 9- NO_2 -A forms 9,10-anthraquinone, both in solution and on silica gel (75) [Eq. (16)]. Furthermore, 6- NO_2 -BaP deposited on silica gel also photolyzes to the expected BaP quinones (1,6, 3,6, and 6,12 isomers) (26).

By analogy one might expect similar photo-oxidations in ambient POM. However, such processes may prove highly dependent on the structure of the nitro-PAH and the nature and treatment of the substrate surface. We are currently

studying such structure-relationships using as a guide the mechanism Chapman developed for 9- NO_2 -A (109). With this nitroarene, a major primary photochemical act is rearrangement into a nitrite, followed by dissociation into NO and a phenoxy-type radical; ultimately the quinone is formed.



If one applies Chapman's elegant mechanistic theory to a more general case, the isomers of nitro-BaP, some interesting predictions can be made. The 6- NO_2 isomer with 2 *peri*-hydrogens should be photochemically less stable than the 1- and 3-isomers with only 1-*peri*-hydrogen (Fig. 12). Indeed, this appears to be the case. 6- NO_2 -BaP photodecomposes so readily in solution that our HPLC separations of this compound are made with the UV analyzer beam off. In contrast, the 1- and 3- isomers with 1-*peri*-hydrogen seem quite stable, at least under these conditions of irradiation (110).

Experiments are currently underway in our laboratory to test the generality of this Chapman

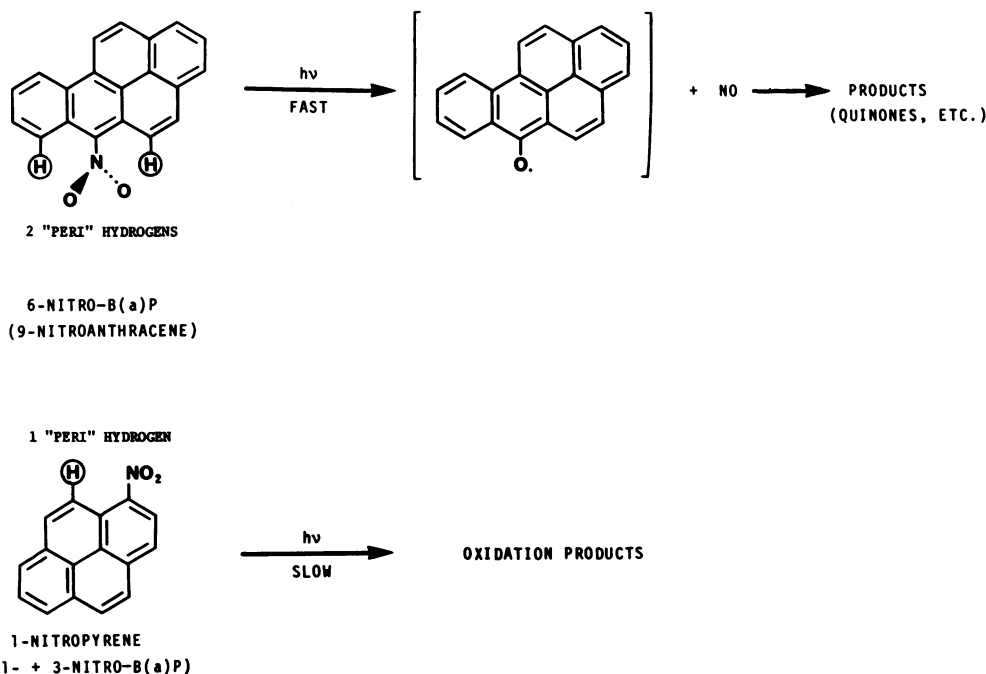


FIGURE 12. Possible effect of the molecular environment of the NO_2 group on the photostability of two nitroarenes.

mechanism with a variety of nitro-PAH in solution and on solid substrates. Possible correlations of their photoreactivities with their mass spectra (26,111) are also being explored [i.e., with their relative efficiencies of the loss of m/e 16 (O atoms) and 17 (OH) under electron impact (102)].

Hopefully, the results of these fundamental studies may prove to have some useful environmental implications. Thus, even from our limited initial studies on the mononitro isomers of BaP, one can infer that during daylight hours 6-NO₂-BaP on the exposed surface of POM should have a much shorter lifetime than the 1- and 3-isomers and ultimately degrade to several quinones. The latter are either very weak mutagens on strain TA 1537 (1,6- and 3,6-isomers) or nonmutagens (6,12-quinone) (112). Conversely, the 1- and 3-nitro isomers of BaP should have relatively longer atmospheric lifetimes, at least with respect to photodegradation (110).

Furthermore, these results also illustrate the need for proper storage and handling of the nitro-PAH used for long-term animal studies, especially those which are very photolabile, such as 6-NO₂-BaP.

Nitroquinones. Since quinones are among the more stable products of photooxidation of nitro-PAH (and the ozonolysis and photooxidation of PAH themselves), it is interesting that 2-methyl-1-nitro-9,10-anthraquinone (2-methyl-1-nitroanthraquinone) produces tumors when included in the diet of rats and mice (113). Thus it seemed environmentally relevant to attempt to nitrate several representative quinones by exposure to NO₂ in air, even though the rates were expected to be slow relative to the parent PAH because of the electron-withdrawing properties of the carbonyl groups in the quinone structure.

Three representative quinones, anthraquinone, benz(a)anthracene 7,12-dione and benzanthrone, were deposited on filters and exposed in the dark for 19 hr to 1 SCFM of air containing 0.45–1.00 ppm NO₂. They were also exposed in parallel in the dark to air without the NO₂. Work is still in progress, but preliminary results indicate a failure under these conditions to nitrate significant amounts of any of the three quinones or to change significantly their mutagenic activities. For example, the anthraquinone exposure products yielded no activity to strain TA 98 up to 100 µg/plate (102). However, it is worth considering the possibility that nitroquinones may be formed by other routes (e.g., photooxidation of dinitro-PAH). We are testing this hypothesis.

Direct-Acting and Promutagens: Oxidized PAH

In addition to nitroarenes, a great deal of attention has been given recently to oxidized PAH as

possible contributors to the direct-acting frameshift-type mutagenicity of ambient POM. Historically, much of this current interest dates back to early studies of Kotin et al. on the carcinogenic activity of products of the oxidation of aliphatic hydrocarbons (114) and of ozonized gasolines (115). As noted earlier, subsequent studies demonstrated the carcinogenic and cell transformation activities of polar fractions of organic particulates (5,6,10,15,116,117).

From the outset, interest centered on the photooxidations of PAH (8) with some of the most relevant early results being those of Tebbens et al. who studied the chemical modifications in smoke irradiated while passing through a flow chamber (118). They observed a loss of 35–65% of the BaP and perylene in the original sample. Subsequently, Thomas et al. found a 60% decrease in the BaP content of soot from the entrance to the exit of a 22-ft long irradiation chamber (119). However, neither the PAH reaction products nor their biological activities were established.

In 1976, several phenols and epoxides known to be metabolites of BaP in mammalian cells were shown to be direct-acting frameshift mutagens (120–123). In part by analogy with these metabolites, it was suggested that certain of the direct mutagens in ambient POM could be formed in atmospheric reactions of BaP (and other PAH) with pollutants present in photochemical smog such as O₃ and PAN (and NO₂), as well as in PAH photooxidations involving singlet molecular oxygen (O₂¹Δ) and the free radicals OH and HO₂ (26,74).

Combined chemical/microbiological studies of BaP exposed to the gaseous portion of filtered ambient photochemical smog, as well as to low levels of O₃ and PAN (and NO₂) in pure air, confirmed that direct-acting frameshift mutagens were indeed formed, some with TLC retention times and mass spectra resembling certain of the BaP metabolites formed when BaP was treated with S9 (75).

The present situation, with regard to the degradation of PAH by actinic light and/or ozonolysis, can be summarized briefly as follows (2,5,74,78).

Degradation of PAH. Polycyclic aromatic hydrocarbons can be photooxidized in both simulated and ambient atmospheres when either pure PAH, synthetic mixtures or ambient POM are irradiated with actinic UV light. Additionally, certain PAH such as BaP can be readily oxidized by exposure to ambient levels of ozone in simulated and real atmospheres; others are far less reactive (24,26,74,75,126–129).

The quantum yield of photodegradation depends on the structure of the PAH and the physical and chemical nature of the substrate upon which it is deposited. Thus adsorption on fly ash appears to

stabilize certain PAH towards photodecomposition (130). On the other hand, many PAH readily photooxidize when deposited on carbon black and irradiated (101,102) or when soot containing the PAH is irradiated (118). Additionally, it has been reported recently that certain PAH can degrade in the dark in clean air when deposited on certain substrates (131).

Experimental and modeling studies of the deposition velocities of combustion-generated fine particles such as primary diesel POM (132) show that they (as well as ambient POM) can remain suspended in polluted air for hours and even days, so that ample time exists for the occurrence of chemical and physical, and thermal and photochemical, transformations of surface-bound PAH.

In this regard, recent quantitative measurements of the quantum yields of the heterogeneous photo-degradation of selected PAH deposited on silica gel and exposed to simulated actinic UV light are of interest (133). Absolute quantum yields ranged from 8500×10^{-5} for anthracene to 5.3×10^{-5} for BaP. However, when the differences in absorption cross sections are factored into the estimation of mean tropospheric lifetimes, the values become 0.02 vs. 10 hr, respectively, for this particular mode of decomposition. Of course, other competing processes (e.g., ozonolysis) could change these relative and absolute values. It should be noted that long-range transport studies of POM during periods of low light intensities and low ozone levels did not show appreciable degradation of PAH. This seems reasonable since photooxidative processes would be expected to be at a minimum (134,135).

A variety of products are formed on ozonolysis and/or direct photooxidation of PAH on solid substrates. However, with the possible exception of anthracene, the nature of the products, their rates of formation, the detailed reaction mechanisms and the structures of the reactive intermediates are not adequately understood—indeed in most cases they are simply not known.

Obtaining a good understanding of “real world” PAH photooxidations (e.g., effects of substrates, spectral distribution and light intensity, temperature and RH, etc.) obviously is an even more complex and demanding task than in simulated atmospheres. However, it is an essential one if the chemical dose inputs to risk-benefit assessment calculations on the health and other environmental impacts of combustion-generated POM is to have reasonable validity.

Clearly, present research efforts in this field, both on the fundamental and applied side, should be strengthened and expanded—but only on the basis that the chemistry and photochemistry involved be conducted at the highest level of imagination and

execution. The time has arrived for far more quantitative studies of the rates, products and mechanisms of these complex heterogeneous reactions—even though by their very nature they will be difficult, expensive and time-consuming research tasks.

Mutagenicity of Oxidized PAH. While some products from the photooxidation and ozonolysis of PAH in simulated and real atmospheres have been identified (completely or tentatively), to date most of these have been quite stable chemically and have not proven to be direct-acting mutagens in the Ames assay (or at best only very weak). Examples include the spectrum of quinones generated from tricyclic-pentacyclic PAHs. Similar considerations apply to PAH oxygenates in primary POM from diesel or spark engine exhausts and the organic fractions from fly ash.

This is not surprising. Thus, by analogy with those BaP metabolites which are most active in this assay (e.g., diol-epoxides), one might expect the moderate to strong directly active mutagenic species in “environmentally” oxidized PAH to be quite unstable chemically. Thus they could be readily transformed into more stable, nonmutagenic compounds by atmospheric reactions, or during sampling and subsequent separation and analysis procedures.

EPOXIDES: While it had been known since 1978 that extracts from BaP deposited on a glass fiber filter and exposed to sub-ppm levels of O_3 showed direct frameshift activity, it took several years and the development of special HPLC separation procedures to isolate the major active species (129). Thus, Ames assays of initial HPLC separations with a methanol-water solvent system had indicated that the activity resided in the fractions containing the 1,6-, 3,6- and 6,12-BaP quinones. However, though major ozonolysis reaction products, these compounds were known to be only slightly mutagenic at best.

Therefore, a new separation procedure employing an acetonitrile-water solvent system was developed; this yielded the “direct mutagenicity vs. HPLC fraction” data shown in Figure 13a. The strongly mutagenic fraction (No. 13) was subjected once more to fractionation and the activity proved to be localized in one band. Spectroscopic and GC-MS analysis (and confirmation with a synthesized authentic sample) showed that the active component was BaP-4,5-oxide. This compound is a well-known BaP metabolite, a powerful direct-acting frameshift mutagen (see Fig. 13b) and a weak carcinogen on mouse skin (136). Our experimental value of ~ 1600 rev/ μg (with TA 98) proved to be in good agreement with those of other researchers (123,137).

While it is tempting to extrapolate these results

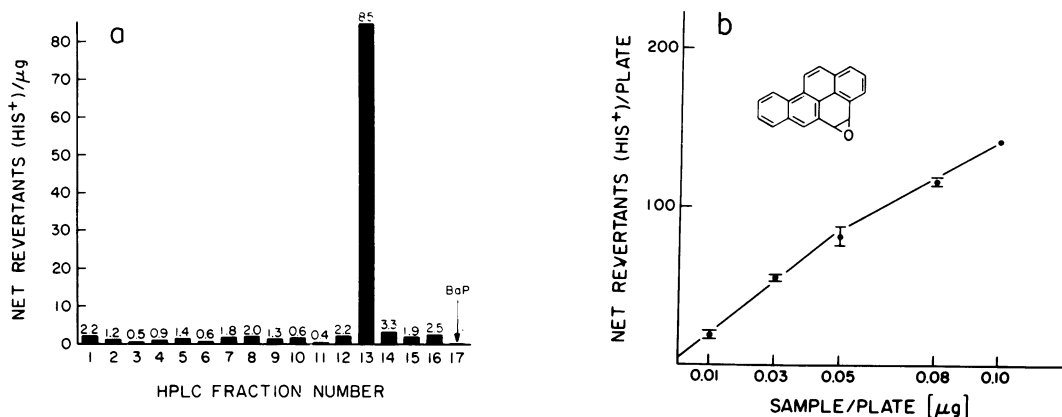


FIGURE 13. Determination of mutagenic product(s) of BaP-O₃ dark reaction: (a) specific mutagenic activity of HPLC fractions of BaP-O₃ products toward TA 98, without activation; (b) dose-response curve for fraction 13-4 (TA 98) without activation.

from a synthetic atmosphere to real-world situations, and while some experimental studies of diesel POM have suggested the presence of epoxides (138), this should be done only with caution. Thus the epoxide yield, while already low for O₃ exposure in the dark (0.5%), is much less when the BaP-coated filter is exposed concurrently to actinic light [0.07% (129)].

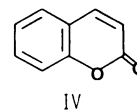
Just how this photo-instability (and other possible liquid-phase reactions on the surface of wet ambient POM) will affect the atmospheric half-life of this arene oxide is not known. Furthermore, the generality of the ozonolysis reaction by which this *K*-region epoxide is formed may well be limited. Thus while under our reaction conditions on glass fiber surface, the general order of reactivity for both photooxidation and ozonolysis processes is BaP > BaA > pyrene > fluoranthene >> perylene; this may not be true for other types of oxidative processes (101,102).

Finally, the efficiency of the reaction is substrate dependent. Thus, the yield of BaP-epoxide from BaP deposited on carbon fiber surfaces is less than on glass. Furthermore, as noted earlier, while NO₂ added to the inlet air of a hi-vol sampler produced significant loss of BaP (60%) in the ambient POM samples collected, similar "doping" of inlet ambient air with 200 ppb of O₃ had no effect (99).

LACTONES: This class of PAH derivatives is especially interesting because we have now identified at least one member, 4-oxapyrene-5-one (I), (Fig. 14) both as a product of the photooxidation of pyrene under simulated atmospheric conditions and as a mutagenic constituent of diesel (90) and ambient POM (102). While this lactone is not directly mutagenic, as we reported in an earlier publication (90), it does exhibit promutagenicity toward strain TA 100 (Table 4). Thus, formation of this compound through photooxidation of pyrene in ambient air

would represent the chemical transformation of a nonmutagen to a mutagenic product.

Preliminary evidence from our heterogeneous surface photooxidations of other PAH (e.g., BaP) suggests that lactone formation may be a general phenomenon. This is of some interest since a BaP analog (II, Fig. 14) is reported to be a highly active promutagen (140). Furthermore, the photooxidation of phenanthrene has been reported (141) to give benzocoumarin (III). Finally, these *K*-region lactones are related to coumarin (IV), a compound which has been widely used in perfumes and as a flavoring agent (142). However, recently it has been described as a weak carcinogen in mice (143,144).



It would appear that this class of compounds needs more detailed investigation, including the mechanisms of formation, yields and biological activities.

OTHER MUTAGENIC OXIDIZED PAHS: As indicated at the outset, there is a dearth of reliable information (i.e., isolation, proof of structure, etc.) about other possible PAH oxygenates that may be mutagenic. However, the data base is improving.

For example, recent studies of the photooxidation of anthracene dispersed into ambient POM suggest the involvement of O₂(¹Δ) as a major environmental process with this PAH (145). Indeed, the possibility of ambient PAH acting both as photosensitizers to form O₂(¹Δ), and as receptors to form oxidized products, was suggested some years ago (73) and may have some generality (5,146-149).

In this regard, recent experiments in which photodynamically generated O₂(¹Δ) was reacted with chrysene and 3-methyl-cholanthrene to form prod-

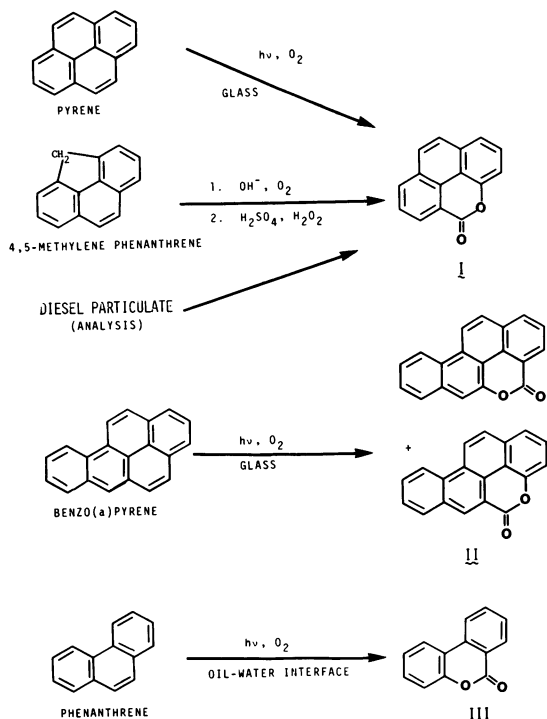


FIGURE 14. Structures of some environmentally relevant K-region lactones.

ucts that were direct-acting mutagens seem highly relevant to the entire question of possible mechanisms of atmospheric oxidations of POM to produce biologically active products (150).

The report of Rappaport et al. on the anhydride derivative of pyrene isolated from diesel POM (139) is also important. Thus, although this specific compound is only a very weak direct mutagen in the Ames bacterial assay, it, or other members of this class of compounds, may prove to be far more active in other *in vitro* or *in vivo* test systems. Similar considerations apply to other oxygenated (e.g., lactones) and nitrated (e.g., 3-NO₂-perylene and 6-NO₂-BaP) PAH derivatives. For example, some of these show only weak to moderate or no direct activity with the Ames Salmonella reversion assay system but are powerful promutagens and possible carcinogens.

In conclusion, it seems that from a health effects perspective, we atmospheric chemists must keep in mind that membership of a PAH derivative in the bacterial "Mutagen-of-the-Month Club" is an important, but not necessarily sufficient, criterion for concern. Indeed, as biomedical researchers well recognize, it should be only the first step to a spectrum of other *in vitro* and *in vivo* tests culminating in animal carcinogenicity evaluations and, when appropriate, associated epidemiological studies.

From an overall ecological perspective it is important to keep in mind that degradation of PAH through various oxidative mechanisms yields polar products that have greatly increased solubilities in aqueous systems. For example, transport of these PAH derivatives across phase boundaries from the surfaces of ambient particles into rivers, lakes, underground aquifers and the ocean will be more efficient than for the original polycyclic aromatic hydrocarbons.

This paper is based on current research at the Statewide Air Pollution Research Center and the Department of Chemistry, University of California, Riverside. Dr. D. Lokensgard, Mr. W. Harger and Mr. D. Fitz were leaders in the research on the transport and transformation of POM while Drs. G. Harris, E. Tuazon and A. Winer from UCR and Drs. U. Platt and D. Perner from Jülich led the long path spectroscopic studies of noncriteria pollutants. My special thanks to them and the other members of the SAPRC-Chemistry team, including Drs. R. Atkinson and W. Carter, for their dedicated efforts.

I also appreciate stimulating discussions with Drs. A. Bjørseth and B. J. Finlayson-Pitts, and the excellent editing assistance of Ms. Y. Katzenstein, Ms. M. Peterson and Ms. L. Sanchez, manuscript preparation by Ms. M. Minnich and Ms. C. Ranck, and statistical analyses by Ms. M. Poe. Additionally the generosity of Professors B. Ames and H. Rosenkranz who supplied the Salmonella strains used in this research, and Dr. Robert Mermelstein, who provided us with several nitroarene samples, is deeply appreciated.

My thanks also go to the University of California and to the following agencies that have generously supported research in the areas discussed in this paper: State of California Air Resources Board (Drs. J. Holmes and J. Suder); U. S. Department of Energy (Drs. D. Ballentine, F. Hudson and G. Stapleton); National Science Foundation (Dr. R. Carrigan); and the Environmental Protection Agency (Drs. P. Hanst and R. Papetti and Mr. B. Gay). The contents of this paper do not necessarily reflect the views and/or policies of these agencies nor of the University of California, Riverside.

Finally, I wish to thank Professors L. Friberg and B. Holmberg, the other members of the Organizing Committee, and the Karolinska Institute for the opportunity to participate in this Symposium.

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